

PHYSIOLOGICAL STUDIES ON THE GERMINATION OF *ERYTHRINA CAFFRA* THUNB. SEEDS

J. G. C. SMALL, JUDITH E. McNAUGHTON AND J. H. GREEFF

(Department of Botany, University of Port Elizabeth)

ABSTRACT

Dormancy of *Erythrina caffra* seeds was overcome by mechanical abrasion, piercing of the hilum or sulphuric acid scarification. Heat treatments were ineffective.

Germination was not affected by light. The optimum temperature for germination was 30 °C.

The testa restricted oxygen uptake. The very high RQ-values of germinating seeds was reduced by testa removal or increased oxygen tension. Acid scarified seeds had higher germination rates than abraded or hilum pierced seeds. This was attributed mainly to an increased oxygen permeability of the testa.

UITTREKSEL

ONTKIEMINGS FISIOLOGIE VAN *ERYTHRINA CAFFRA* THUNB. SAAD

Die rustoestand van *Erythrina caffra* saad is opgehef deur die saadhuid te skuur, 'n gaatjie in die hilum te steek of deur swaelsuurweking. Hitte behandelings was oneffektief.

Ontkieming is nie deur lig beïnvloed nie. Die optimum ontkiemingstemperatuur was 30 °C.

Die testa beperk suurstofopname. Die hoë RK-waardes van ontkiemende saad kon deur testaverwydering of verhoogde suurstofspanning, verlaag word. Suur behandelde saad het vinniger ontkiem as geskuurde of hilum deurboorde saad. Dit is hoofsaaklik daaraan toegeskryf dat swaelsuur die deurlaatbaarheid van die testa vir suurstof verhoog.

INTRODUCTION

Seeds of *Erythrina caffra* fail to germinate when incubated under conditions normally favourable for germination. The dormancy may be expected to be due to impermeability of the seed coat to water as this is a common feature of the family Leguminosae (Hamly, 1932; Barton, 1965; Villiers, 1972; Mayer & Poljakoff-Mayber, 1975). Impermeability of the seed coat to water in this family has been ascribed to the cells of the palisade layer (Esau, 1967; Villiers, 1972). The testa of *Erythrina caffra* has a well defined palisade layer as shown by McNaughton & Robertson (1977).

This investigation describes experiments on the effect of scarification method, heat treatment, light and temperature on germination. Water uptake, seed respiration and the effect of oxygen and nitrogen on germination were also studied in an effort to explain differences in germination rate caused by different scarification methods.

MATERIAL AND METHODS

Seed material

Seeds were collected from a single tree growing in Nelson Square, Port Elizabeth. Healthy seeds of uniform size, lacking beetle sting and other foreign markings, were selected. These were treated with phostoxin (Bayer Agro-Chem) to prevent accumulation of and attack by beetles, aerated briefly and stored in stoppered glass jars in the laboratory. The phostoxin treatment had no effect on subsequent germination.

Germination

Depending on the experiment, seeds were germinated on three layers of filter paper in 90 mm glass petri dishes, or in 500 ml conical flasks or in washed river sand. Twenty-five seeds were used per replicate. Petri dishes contained 20 ml of distilled water and conical flasks 25 ml. These water levels were found in preliminary experiments to be optimal for germination. The sand was kept moist. Petri dishes were oven sterilized; conical flasks, sand and water were autoclaved.

Seeds were surface sterilized by immersion in 70 % ethyl alcohol for one minute followed by 4 minutes in 0.1 % HgCl_2 and subsequently rinsed thoroughly with sterile, distilled water. This method was found to be more effective than hypochlorite sterilization. In all cases seeds were placed at random in the germination medium, i.e. not orientated in a particular way.

Germination tests were performed in Controlled Environments Model E7H growth cabinets maintained at 80 % RH. Treatments were replicated 4 times. Radicle emergence was used as the index of germination.

Respiration

Oxygen uptake and carbon dioxide evolution by germinating seeds were determined manometrically using the "direct method" of Warburg (Umbreit, Burris & Stauffer, 1972) in a Gilson differential respirometer equipped with an all glass submarine volumeter system. Measurements were made at 25 °C on 8 replicates of 5 seeds per respiration flask. On completion of gas exchange measurements, seeds were oven dried at 105 °C for 24 hours for dry mass determinations.

RESULTS

Effect of scarification and heat treatment on germination

The following treatments were applied

1. Control—no treatment
2. Mechanical abrasion—seeds were rubbed on one side with sand paper to expose approximately 4 mm² of cotyledon

3. Acid scarification—seeds were soaked for 30 or 120 minutes in concentrated sulphuric acid and then washed in running tap water
4. Hilum pierced—a single hole was pierced through the centre of the hilum with a dissecting needle
5. Dry heat—seeds were placed in an oven at 90 °C for 15 or 30 minutes
6. Hot water—seeds were immersed in hot water (80 °C and 100 °C) for 10 seconds or 5 minutes.

After treatment all seeds were surface sterilized and incubated in the dark at 25 °C in petri dishes. The number of swollen and germinated seeds were recorded after seven days (Table 1).

The highest percentages of swollen and germinated seeds were obtained after mechanical abrasion, acid scarification for 120 minutes and hilum piercing. Although some of the heat treatments did cause an appreciable number of seeds to swell, they were extremely detrimental to germination.

A small number of control seeds germinated. On closer inspection it was found that these seeds had minute holes in their seed coats. It was later confirmed that a small beetle (*Bruchidius* species) was responsible for puncturing and parasitizing *Erythrina* seeds.

Effect of light and temperature on germination of 120 minute acid scarified and mechanically abraded seeds

Seeds were incubated in petri dishes in the light and in the dark at 15 °, 20 °, 25 °, 30 ° and 35 °±0.5 °C. Lighting was obtained from fluorescent tubes at an intensity of approximately 3 800 lux. The dark treatment was obtained by wrapping petri dishes in one layer of aluminium foil. Germination counts of dark-treated seeds were performed under "safe" green light.

Similar germination counts were obtained in the light and in the dark. No interaction between light and seed coat treatment, temperature and seed coat treatment, or between light and temperature was observed. However, temperature markedly affected germination. The optimum temperature for germination was found to be 30 °C. This is shown in Fig. 1 in which the mean germination counts after 48 hours of incubation are plotted.

During this, and subsequent experiments it was noted that acid treated seeds had a higher germination rate than abraded seeds. The slower germination of the latter was not simply a function of abraded area since seeds sandpapered to expose approximately 1 mm², 4 mm² and 12 mm² had similar germination rates. The "acid effect" was therefore investigated further.

Effect of scarification method on water uptake

To test whether the stimulatory effect of acid scarification was due to an increased water uptake, the moisture content of abraded, acid scarified (120

TABLE 1
Effect of scarification and heat on swelling and germination after 7 days incubation
(means of 4 replicates $\pm$ SE).

	Control	Mech Abraded	Hilum pierced	TREATMENTS				Hot water 80 °C		Hot water 100 °C	
				H ₂ SO ₄		Dry heat 90 °C		10 sec	5 min	10 sec	5 min
				30 min	120 min	15 min	30 min				
% Swollen	4 $\pm$ 1,6	100 $\pm$ 0	94,6 $\pm$ 5,3	37 $\pm$ 3,4	99 $\pm$ 1,0	37 $\pm$ 9,4	63 $\pm$ 3,4	10 $\pm$ 3,4	33 $\pm$ 5,2	8 $\pm$ 1,6	67 $\pm$ 7,7
% Germinated	4 $\pm$ 1,6	92 $\pm$ 8	94,6 $\pm$ 5,3	30 $\pm$ 4,7	95 $\pm$ 2,5	0	0	10 $\pm$ 3,4	9 $\pm$ 3,8	6 $\pm$ 2,0	0

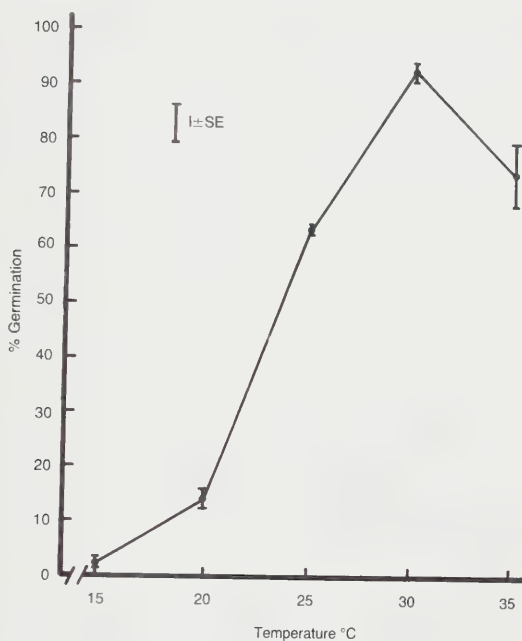


FIG. 1.

Effect of temperature on germination after 48 hours incubation.

minutes) and hilum pierced seeds was compared over a 96 hour period of incubation. Three germination techniques were used, viz. germination in sand, in petri dishes and in conical flasks stoppered with cotton wool.

Incubation was at 25 °C in the dark. After various periods three replicates of 25 seeds each were removed for germination counts and moisture determination. Moisture content was calculated on a fresh weight basis after oven drying at 105 °C for 24 hours.

Similar results were obtained with the different germination techniques. The effect of seed coat treatment on rate of germination and moisture content of seeds germinated in petri dishes is shown in Fig. 2.

As previously found, sulphuric acid scarification resulted in greater germination rates than either mechanical abrasion or hilum piercing. Water uptake, however, was slower after acid scarification. These results suggest that the beneficial effect of sulphuric acid scarification was not caused by increased water uptake.

It was noted that water uptake (judged by swelling pattern) of acid scarified

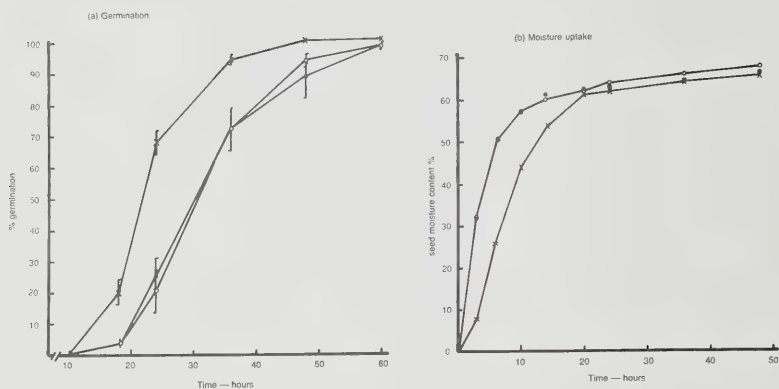


FIG. 2.

Effect of seed coat treatment on germination and moisture uptake (X, H₂SO₄ scarified; ●, Hilum pierced; ○, Abraded).

and hilum pierced seeds proceeded from the hilum area whereas swelling of abraded seeds proceeded from the abraded area.

Respiration studies

Although the impermeability of the seed coat to water is undoubtedly the cause of dormancy in *E. caffra* it was thought possible that the different seed coat treatments could affect oxygen permeability differently and thus cause differences in germination rate. It is known that, apart from affecting water uptake, scarification may induce other changes also, such as permeability to gases, changes in sensitivity to light or temperature and possibly even destruction or removal of inhibitory substances (Mayer and Poljakoff-Mayber, 1975).

To test whether the seed coat restricted gaseous exchange, the respiration of abraded seeds which had imbibed water for 18 hours was measured in air and compared with that of similar seeds allowed to respire in pure oxygen and to that of embryos in air after removal of the testa. The results are shown in Table 2.

TABLE 2

Effect of seed coat removal and oxygen on respiration of seeds after 18 hours imbibition.

Seed Treatment	O ₂ uptake $\mu\ell\ g^{-1}\ hr^{-1}$	CO ₂ evolution $\mu\ell\ g^{-1}\ hr^{-1}$	RQ
Abraded, respiring in air	10,8±0,2	76,2±4,4	7,06±0,4
Abraded, respiring in oxygen .	50,3±1,7	68,4±1,5	1,36±0,03
Minus seed coats, respiring in air	122,3±11,3	73,2±2,9	0,6±0,02

An increase in oxygen uptake, and drop in RQ-value were observed when seeds respired in pure oxygen or when testas were removed. The greatest effect was caused by testa removal. These results demonstrate that the seed coat, in spite of abrasion in a small area, markedly restricts oxygen uptake.

To test whether acid scarification affects the permeability of the seed coat to oxygen differently to mechanical abrasion, the respiration of seeds was determined after various periods of incubation at 25 °C in conical flasks stoppered with cotton wool.

The results are shown in Fig. 3.

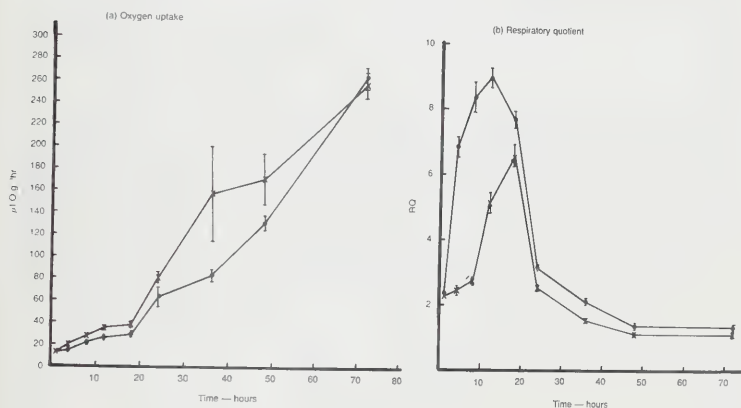


FIG. 3.

Effect of scarification on respiration (● Abraded; X, H_2SO_4 scarified).

Up to about 65 hours after the commencement of imbibition, the rate of oxygen uptake was higher in acid scarified than in abraded seeds. The RQ-values of abraded seeds were initially of a much higher order than that of acid treated seeds.

Effect of oxygen and nitrogen on germination

From the results of the previous experiment it appeared that acid scarified seeds were more permeable to oxygen than abraded seeds. It could therefore be expected that increasing the oxygen partial pressure would reduce differences in germination rate. This hypothesis was tested by germinating acid scarified and abraded seeds in air and in pure oxygen in conical flasks at 25 °C. For air treatment, flasks were stoppered with cotton wool. To obtain the oxygen treatment, flasks were stoppered with vaccine caps through which two hypodermic needles were inserted. High purity oxygen was passed through the flasks at a rate

of 500 ml per minute for 15 minutes after which the gas flow was stopped. The hypodermic needles were removed after allowing a short period for flask and atmospheric pressure to equilibrate.

In addition, seeds were incubated in nitrogen at atmospheric pressure. This was obtained by repeated evacuation and refilling vaccine capped conical flasks containing seeds, with high purity nitrogen following the method of Umbreit *et al.* (1972).

As found previously, acid treated seeds germinated faster than abraded seeds when incubated in air (Fig. 4). An initially high concentration of oxygen increased the germination rate of abraded seeds but not that of acid scarified seeds. A high nitrogen concentration retarded germination; the effect being similar for both seed coat treatments.

DISCUSSION

The results obtained in this investigation have shown clearly that the dormancy of *E. caffra* seeds is caused by a hard testa impermeable to water. Of the methods

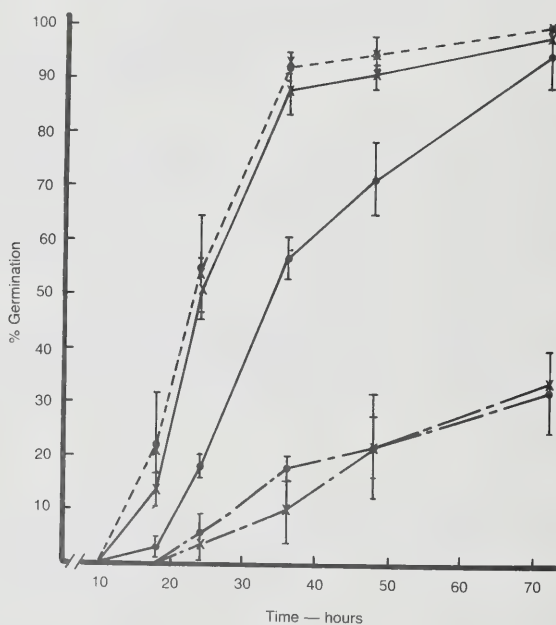


FIG. 4.

Effect of air, oxygen and nitrogen on germination of abraded and acid scarified seeds (● Abraded; X, H₂SO₄ scarified; — Air; - - - Oxygen; — — — Nitrogen).

tested, piercing the hilum, mechanical abrasion and sulphuric acid scarification were most effective in causing water uptake and germination. Although only a limited range of heat treatments were used, the results indicate that *E. caffra* is rather sensitive to heat and that this type of treatment is ineffective in promoting germination in this species. This is concluded from the finding that increased extremity of heat treatment caused an increase in percentage swollen seeds but a decrease in germination percentage. Various authors have used heat as a treatment to soften seeds of a number of species (Barton, 1965). Although high germination can be obtained with heat treatment in some cases, (e.g. Gray, 1962) it appears that there is a considerable variation in germination response to heat among legume genera and species (Martin, Miller & Cushura, 1975).

The extreme "hardness" of *E. caffra* seeds poses the question of how these seeds are rendered permeable to water in nature. It is generally assumed that the most common way in which such seeds become permeable in nature is through attack by micro-organisms. Both Barton (1965) and Villiers (1972) state that while bacterial or fungal action is very probably responsible for surface deterioration of seeds, evidence for this is scarce. In the case of *E. caffra* it is possible that a puncturing of the seed coat by a *Bruchidius* species could contribute to dormancy breaking in a small percentage of seeds. If sufficient moisture were available it appears probable that at least some parasitized seeds could germinate before being killed by the developing larvae.

The temperature effects found and the relatively high optimum temperature (30 °C) for germination, correlates with the fact that *E. caffra* inhabits warm subtropical regions. The lack of a light effect on germination is in accord with the behaviour of several legume species (Nakamura, 1962).

Apart from the impermeability of the testa to water it also appeared to restrict oxygen diffusion. Evidence for this was found in the lowered RQ-values when seeds respired in pure oxygen or in air, after removal of testas. Furthermore, an increased oxygen tension increased germination rate. The extremely high RQ-values suggest that fermentation had taken place. This is a common feature of the early stages of germination (Mayer & Poljakoff-Mayber, 1975). The respiratory pattern observed was similar to that found by Spragg & Yemm (1959) for peas although they recorded much lower RQ-values.

Throughout this study it was consistently found that sulphuric acid-scarified seeds germinated at a higher rate than mechanically abraded seeds. This was not due to an increased water uptake. Also, it did not appear to be due to a weakening and thus a decrease of the resistance of the testa to radicle emergence, since the seed coat treatments resulted in equal rates of germination in a high nitrogen atmosphere. Oxygen permeability, however, appeared to have been enhanced by acid treatment. This was seen in the higher rate of oxygen uptake and lower order of RQ-values of acid treated seeds compared to abraded seeds. A decrease in RQ-value would indicate a decrease in fermentative activity. Although the respira-

tory activity of seeds of both seed coat treatments contained a high component of fermentation during the early germination stages, it is concluded that the stimulatory effect of acid treatment was due to decreased fermentation and thus to an increase in the aerobic component of respiration.

The mode of action of sulphuric acid in scarification is not clear. Burns (1959) showed that sulphuric acid dissolved the counter palisade layer which obstructs the hilar fissure in *Lupinus angustifolius* or eroded pits through the testa. In the present study it was found that the hilar region was affected most, as judged by the swelling pattern of the seeds. Whether the increased oxygen permeability was restricted to this area was not ascertained.

ACKNOWLEDGEMENTS

The beetle, parasitizing *Erythrina* seeds, was identified by Dr J. P. Furstenberg, Department of Zoology, University of Port Elizabeth.

Financial assistance was received from the University of Port Elizabeth and the C.S.I.R., Pretoria.

REFERENCES

- BARTON, Lela V., 1965. Dormancy in seeds imposed by the seed coat. In: W. Ruhland, (ed.), *Encyclopedia of Plant Physiology*. Vol. 15 (2). Berlin, Heidelberg, New York: Springer-Verlag. p. 727-745.
- BURNS, R. E., 1959. Effect of acid scarification on lupine seed impermeability. *Pl. Physiol., Lancaster* **34**: 107-108.
- ESAU, K., 1967. *Anatomy of Seed plants*. New York: Wiley.
- GRAY, S. G., 1962. Hot water seed treatment for *Leucaena glauca* (L.) Benth. *Aust. J. exp. Agric. Anim. Husb.* **2**: 178-180.
- HAMLY, D. H., 1932. Softening of the seeds of *Melilotus alba*. *Bot. Gaz* **93**: 345-375.
- MARTIN, R. E., MILLER, R. L., and CUSHWA, C. T., 1975. Germination response of legume seeds subjected to moist and dry heat. *Ecology* **56**: 1441-1445.
- MAYER, A. M. and POLJAKOFF-MAYBER, A., 1975. *The Germination of Seeds*. 2nd ed. New York: Pergamon.
- MCNAUGHTON, J. E. and ROBERTSON, B. L., 1977. A note on the seed-coat structure of *Erythrina caffra* Thunb. *Jl S. Afr. Bot.* **43**: 209-211.
- SPRAGG, S. P. and YEMM, E. W., 1959. Respiratory mechanism and the changes of glutathione and ascorbic acid in germinating peas. *J. exp. Bot.* **10**: 409-425.
- UMBREIT, W. W., BURRIS, R. H. and STAUFFER, J. F., 1972. *Manometric and Biochemical Techniques*. 5th ed. Minneapolis: Burgess.
- VILLIERS, T. A., 1972. Seed dormancy. In: T. T. Kozlowski, (ed.), *Seed Biology*. Vol. 2. New York and London: Academic Press. p. 219-281.